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Behavioural plasticity in the onset of dawn song under intermittent experimental night lighting

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The disruption of daily rhythms is one of the most studied ecological consequences of light pollution. Previous work showed that several songbird species initiated dawn song earlier in areas with light pollution. However, the mechanisms underlying this shift are still unknown. Individuals may immediately adjust their timing of singing to the presence of artificial light (behavioural plasticity), but the observed effect may also be due to phenotype-dependent habitat choice, effects of conditions during early life or micro-evolution. The main aim of this study was to experimentally investigate how males of four common passerine species respond to day-to-day variation in the presence of artificial night lighting in terms of the timing of singing. During two consecutive breeding seasons, we manipulated the presence of light throughout the night in a cyclic fashion in several naturally undisturbed forest patches. We show that individuals of all four species immediately and reversibly adjusted their onset of dawn singing in response to artificial light. The effect was strongest in the European robin, but relatively small in the blue tit, the great tit and the blackbird. The effect in the latter two species was smaller than expected from the correlational studies. This may be coincidence (small sample size of this study), but it could also indicate that there are longer-term effects of living in light-polluted urban areas on timing of dawn singing, or that birds use compensatory behaviours such as light avoidance. We found no evidence that our light treatment had carryover effects into the subsequent dark period, but robins progressively advanced their dawn singing during the light treatment.

The singing behaviour of many songbird species is characterized by a peak of song production around sunrise, i.e. the dawn chorus, and previous work suggests that the timing of dawn song initiation may be regarded as a reliable predictor of male quality (Grava, Grava, & Otter, 2009; Murphy, Sexton, Dolan, & Redmond, 2008; Otter, Chruszcz, & Ratcliffe, 1997; Poesel, Kunc, Foerster, Johnsen, & Kempenaers, 2006). Several studies suggest that artificial night lighting advances the start of the dawn chorus in songbirds (Da Silva, Samplonius, Schlicht, Valcu, & Kempenaers, 2014; Dominoni, Carmona-Wagner, Hofmann, Kranstauber, & Partecke, 2014; Kempenaers, Borgström, Loës, Schlicht, & Valcu, 2010; Miller, 2006; Nordt & Klenke, 2013), potentially affecting their reproductive success (Kempenaers et al., 2010). However, these studies are all correlational and the mechanisms underlying this effect remain unknown.

In general, four nonmutually exclusive mechanisms can explain the differences in timing of behaviour observed between birds living in artificially illuminated environments and those inhabiting naturally dark places (Partecke, 2014; Swaddle et al., 2015). (1) Phenotypic plasticity in the form of behavioural flexibility: this involves an immediate adjustment of the behaviour and generally facilitates adaptive responses to changes in the environment (Price, Qvarnström, & Irwin, 2003; Sih, Ferrari, & Harris, 2011). Behavioural plasticity may be particularly important for coping with anthropogenic changes (Hendry, Farrugia, & Kinnison, 2008; Sih et al., 2011). It is the most likely mechanism, because the initiation of dawn singing is strongly linked to levels of natural light (Hutchinson, 2002) and depends on the light sensitivity of the species (Berg, Brumfield, & Apanius, 2006; Thomas, Kelly, & Goodship, 2004; Thomas et al., 2002). Later start of dawn singing on cloudy mornings (Bruni, Mennill, & Foote, 2014; Da Silva et al.,

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2014; Dominoni et al., 2014; Hasan, 2010; Nordt & Klenke, 2013; York, Young, & Radford, 2014) and earlier singing on mornings with bright moonlight (Bruni et al., 2014; York et al., 2014) also suggest a plastic response to natural light levels. (2) Phenotypic plasticity in the form of developmental effects (Senner, Conklin, & Piersma, 2015): parental phenotype or environmental conditions early in life may influence the development of the trait. For instance, individuals that have been exposed early in life (e.g. in the nest) to artificial light or to early singing males may sing earlier later in life. This requires that individuals that were raised in habitats influenced by artificial night lighting will later also settle and breed there. (3) Phenotypic-based habitat choice: individuals with early chronotypes may preferentially settle in territories affected by artificial night lighting, or 'late' individuals may avoid such areas. (4) Genetic effects: micro-evolution via natural or sexual selection (Diamond, 1986) may have changed the timing of dawn singing for urban birds living in light-polluted areas. This requires limited gene flow between rural birds living in naturally darker habitats and birds living in urbanized, light-polluted environments. Previous work on great tits, *Parus major*, showed that circadian period length is highly heritable and earlier singing may thus result from micro-evolution through selection of fast circadian rhythms (Helm & Visser, 2010).

Here, we report on a study designed to test experimentally whether birds breeding in a naturally dark habitat flexibly adjust their timing of dawn singing in response to artificial night lighting. During two consecutive breeding seasons, we repeatedly exposed songbirds in naturally undisturbed forest patches to a period of artificial light at night, followed by a period of natural darkness. Every morning, we determined the onset of dawn singing of four common songbird species for which an effect of light pollution on timing of singing had been observed previously (Da Silva et al., 2014; Kempenaers et al., 2010). Because the experiment was conducted after territorial settlement, and because we compared the behaviour of individuals under artificial night lighting with that under natural conditions in the exact same location, settlement of individuals with particular chronotypes in the illuminated territories (phenotype-based habitat choice) can be excluded as a potential explanation. Furthermore, developmental and genetic effects can be excluded, because of the short timescale of the experiment. Hence, any effect of the experiment on the timing of dawn singing reflects short-term and plastic adjustments of behaviour in response to the presence or absence of artificial light at night.

The main aim of our study was to test whether artificial night lighting causes birds to start singing earlier at dawn, as suggested by the above-mentioned correlational studies. If behavioural plasticity in response to light is the main (or only) mechanism causing earlier singing, we expect experimental effect sizes that are comparable to those found in the correlational studies. If, however, experimental effect sizes are smaller or there is no effect of the experiment, other mechanisms such as phenotype-based habitat choice may explain the correlational effects. Alternatively, the correlational studies might have been confounded by effects of other causal agents that were correlated with the presence of artificial night lighting.

Environmental light pollution may also be temporary (e.g. during construction works). Hence, a secondary aim of our study was to explore whether a few days of artificial night lighting influence the timing of singing also during subsequent naturally dark nights. Such carryover effects are expected, because the avian circadian system can store information about photoperiod during the days following a transfer from a long day to a short day (and vice versa; Brandstätter, Kumar, Abraham, & Gwinner, 2000; Gwinner & Brandstätter, 2001). If the experimental light had such an effect on the diel rhythm of individuals, we should observe a progressive return to natural times of dawn chorus initiation during consecutive dark days. Such carryover effects have been shown for the timing of emergence from the roost in female blue tits, *Cyanistes caeruleus*, that were experimentally exposed to night light (Schlicht, Valcu, Loes, Girg, & Kempenaers, 2014). We also tested whether the light effect becomes progressively stronger after the control 'treatment' (dark phase), and for simplicity also refer to this as a 'carryover' effect.

METHODS

Experimental Procedure

The experiment was carried out in a woodland area with minimal light pollution in Seewiesen (southern Germany). In 2014, we selected two sites (site 1: coordinates 47.9705°N, 11.2380°E; site 2: 47.9730°N, 11.2325°E). In 2015, we repeated the experiment in the same two sites (although with a small shift for site 1: 47.9708°N, 11.2385°E) plus two additional sites (site 3: 47.97235°N, 11.2363°E; site 4: 47.97435°N, 11.23185°E). The sites were edges of predominantly coniferous forest, mixed with deciduous trees (mainly European beech, *Fagus sylvatica*) and shrubs. They were chosen such that (1) tree density was similar across sites and (2) the following four songbird species were present: European robin, *Erithacus rubecula*, common blackbird, *Turdus merula*, great tit and blue tit. This allowed a direct comparison with descriptive data on the timing of dawn song under artificial night lighting (Da Silva et al., 2014; Da Silva, Valcu, & Kempenaers, 2015; Kempenaers et al., 2010). The experimentally illuminated sites ranged from 0.3 to 0.4 ha and edges of adjacent sites were on average 180 m apart (range 160–200 m). These distances are comparable to the smallest distances between dark control sites and illuminated sites in Kempenaers et al. (2010), where no influence of artificial light on singing in the control areas was detected. Based on these results, we assumed that the singing behaviour of individuals at one site did not influence the singing behaviour of individuals at another site, even though far-carrying songs (e.g. from the robin) could be detected (at lower amplitudes) in the closest neighbouring sites.

At each site, we placed eight mobile halogen spotlights (article number TL800A, Elro, EU) in three parallel rows (two lights in one row, three lights each in two rows) such that lights were approximately 5 m apart. Each light consisted of two bulbs ($N = 16$ bulbs per site), which provided a broad light spectrum (500 W white warm light, colour temperature 2900 Kelvin, flicker rate 50 Hz) at a high intensity (8850 lm, 100 klx at the bulb). Each bulb was directed towards the foliage of the surrounding trees (angle range 20–60°) to illuminate the entire site, from the lower branches to the canopy (and light reflection on the ground). In 2014, we measured an average light intensity of 4 lx during the night at the centre of each site (recorded with a 400 000 Light Lux Meter DT 1308, ATP Instrumentation Ltd, Ashby-de-la-Zouch, U.K., held horizontally 2 m above the ground, 5 m from the nearest light bulb). Ambient light levels during illuminated nights were therefore comparable to intensities found in the centre of large cities, where effects on timing of singing are typically strongest (Dominoni et al., 2014; Nordt & Klenke, 2013).

In 2014, the experiment took place between 1 April and 6 May and consisted of five cycles of an illuminated phase (3 nights with lights turned on from sunset to sunrise), immediately followed by a dark control phase (4 nights without artificial lighting, except for the second cycle where it lasted 5 days by mistake). Dark control phases were longer to allow males to return to their normal singing times after being disrupted by the artificial night lighting (allowing

us to test for carryover effects). In 2015, the experiment ran between 15 March and 1 May. The procedure was the same, with the following modifications. (1) We reduced the dark control phase to 3 days (because no carryover effects were observed in 2014). (2) We arranged the order of the treatments differently between the sites to avoid confounding effects of weather conditions. Thus, in 2015, sites 1 and 2 experienced eight cycles of a dark control phase followed by an illuminated phase, whereas sites 3 and 4 experienced eight cycles of an illuminated phase followed by a dark control phase.

Data Collection

We recorded the dawn chorus using Song Meter SM2+ recorders (Wildlife Acoustics, Concord, MA, U.S.A.), in stereo, with a sampling rate of 22 050 samples/s. In 2014, one recorder was placed in the centre of each site and programmed to record 4 h before until 1.5 h after local sunrise. In 2015, two recorders were placed at the opposite corners of each site (i.e. the top-left and the bottom-right corners) so that the location of song posts relative to the illuminated area could be inferred by comparing song amplitude from the two recordings (see Data Extraction). The two recorders were programmed to record between 4 h before and 1 h after local sunrise. Each sound file was stored as wav-files onto Secure Digital High Capacity cards (Laxer, Fremont, CA, U.S.A.), which were collected every week. A temperature sensor internal to the recorders logged the local temperature every 5 min. To check that the experimental procedure worked properly, a HOBO light sensor (Onset, Bourne, MA, U.S.A.), placed a few decimetres in front of one of the bulbs at each site, recorded light intensity every minute. Lighting malfunctioned during the fourth illuminated phase in site 1 in 2015; we therefore omitted the data from this phase from the analyses.

Study Species and their Presence at the Experimental Sites

In 2015 only, we carried out behavioural observations to obtain information about the location of the territories and about the singing behaviour of individuals of the four study species. Observations took place between 16 February and 27 April ($N = 25$ days), starting between 0504 and 1100 and lasting 10–140 min ($N = 21$ mornings) or between 1240 and 1705, lasting 10–220 min ($N = 8$ afternoons). We used the observations of territorial behaviour in February–March to fine-tune the area we exposed to light based on territory boundaries. At every site, at least one singing male of each species was present, unless otherwise stated; information on the presence of (breeding) individuals of each species at each site is summarized in Table A1 (Appendix). In brief, robins and blackbirds consistently sang at two (2014) and four (2015) sites (for both species: $N_{min} = 4$ individuals, $N_{max} = 6$, depending on whether the same or different individuals were present at site 1 and 2 in the 2 years; $N = 242$ singing days). Great tits sang at two (2014) and three (2015) sites ($N_{min} = 3$ individuals, $N_{max} = 5$; $N = 174$ singing days), and blue tits sang at one (2014) and two (2015) sites ($N = 3$ individuals, $N = 111$ singing days).

Data Extraction

Data were extracted from sound files using Song Scope 4.1.1 (Wildlife Acoustics, Concord, MA, U.S.A.). We used a low-pass filter of 1 kHz, a high-pass filter of 10 kHz and an integrated filter that reduces background noise. On each day and for each site, we noted the time of the first morning song of the four species of interest. We only considered songs for which strophes (i.e. song bursts; Lambrechts & Dhondt, 1988) were repeated at least three times within 5 min, and that were produced prior to sunrise. Extraction rules were slightly different between the 2 study years due to the different protocols. In 2014, we noted the time of the first song that could be distinguished, regardless of its proximity to the recorder (as was previously done in Da Silva et al., 2014, 2015), whereas in 2015, we analysed both recordings from each site in order to infer the position of the singing male relative to the illuminated area. This information, coupled with our behavioural observations, suggested that robins and (to a lesser extent) blackbirds sang at the same or a similar location each day. We therefore visually selected songs that had similar high amplitudes in both recordings, because this indicates that the bird must have sung at or near the illuminated site, hence excluding 'peripheral' individuals that may not have been directly exposed to the artificial light. For great tits and blue tits, we used a different approach, because song posts often varied considerably between days over the experimental period. We differentiated between focal territorial males (i.e. males that sang at the site during most mornings) and peripheral males by inspecting the song characteristics during consecutive mornings. Great tit and blue tits have repertoires consisting of several song types that are sung consecutively by the same individual during the dawn chorus. By visually comparing these song types between mornings we could determine whether the 'typical' (focal territorial) male was singing. Thus, each morning, the first occurrence of a known song type was recorded. We excluded data when the song was hardly audible, i.e. when the territorial male may have been singing far away from the illuminated area (great tit: $N = 14$ recording days; blue tit: $N = 8$ days).

In both years, we noted the presence of rain during the 4 h before sunrise, shown as broad-frequency, low-amplitude, continuous sound bursts. In 2015, a severe storm with strong wind and extended periods of heavy rain occurred from 30 March to 2 April, and this was taken into account in the analyses (see below).

Statistical Analysis

All statistical analyses were performed with the R 3.1.1 software (R Development Core Team, 2014). For each species separately, we used linear mixed-effects models (LMMs fitted by ML, nlme package; Pinheiro, Bates, DebRoy, Sarkar, & the R Development Core Team, 2013) with 'site' (four levels) nested in 'year' (two levels) as a random effect. 'Onset of singing' (in minutes relative to sunrise) was the dependent variable and 'treatment' (light/control), 'date' (intercept = 15 March), 'weather' (clear/rain/storm) and 'temperature at sunrise' (in degrees Celsius) were the explanatory variables. We used a quadratic term for 'date' in the blackbird, great tit and blue tit models because the relationship between onset of singing and date across the breeding season is nonlinear for these species, showing a peak at egg laying (Cuthill & Macdonald, 1990; Mace, 1987). For all the species, we initially compared models that included the interaction between 'treatment' and 'date' with those that did not, based on the Akaike information criterion (AIC) following Burnham and Anderson (2002). We also report the R^2 of each candidate model to assess the predictive quality of the models (Nakagawa & Schielzeth, 2013). Based on ΔAIC_c, none of the models with the 'treatment∗date' interaction was selected as the final model (Table A2, Appendix). We corrected for temporal autocorrelation in all models by using the correlation structure corARMA (Box, Jenkins, & Reinsel, 2013) and for multiple testing within each model using the multcomp package (Westfall, Tobias, Rom,

Wolfinger, & Hochberg, 1999). The fit of the models was assessed by visual inspection of the residuals. 'Onset of singing' was normally distributed for all the species, but for the robin only after log transformation.

We used daily 'temperature at sunrise' because it correlated strongly with daily 'overnight temperature' (defined as the mean temperature during the 4 h prior to sunrise; in 2015: Pearson correlation: $r_{46} = 0.92$, $P < 0.001$). 'Temperature at sunrise' did not correlate with 'date' in 2014 ($r_{34} = 0.18$, $P = 0.28$), and only moderately in 2015 ($r_{46} = 0.34$, $P = 0.02$). Rainy days tended to be on average 2° warmer than nonrainy days in 2014 (Mann–Whitney U test: $U = 100$, $N_1 = 21$, $N_2 = 15$, $P = 0.07$) and 4.8° warmer in 2015 ($U = 66$, $N_1 = 35$, $N_2 = 13$, $P < 0.001$, stormy days were counted as rainy days). In 2014 there were more rainy days during dark phases ($N = 11/21$, 52.4%) than during illuminated phases ($N = 5/15$, 33.3%).

To compare the experimental effect of the light treatment on the onset of dawn singing with the correlational effect reported previously (Da Silva et al., 2014), we evaluated whether the experimental effect fell within the 95% confidence interval (CI) of the previously reported effects. We tested for carryover effects from long days (illuminated nights) to short days (dark control nights) and vice versa using LMMs (fitted by ML, lme4 package; Bates, Maechler, Bolker, & Walker, 2013) by assessing whether the slope in onset of dawn song over the first 3 days of each new phase differed from zero (separately for each treatment type). If birds that enter a new phase are still affected by the previous phase, we expect that the onset of singing will become increasingly later as the dark phase progresses (positive slope), and possibly also earlier with each illuminated night (negative slope; Gwinner & Brandstätter, 2001). We used 'cycle' (from 1 to 5 in 2014, 1 to 8 in 2015) nested in 'site' (four levels),

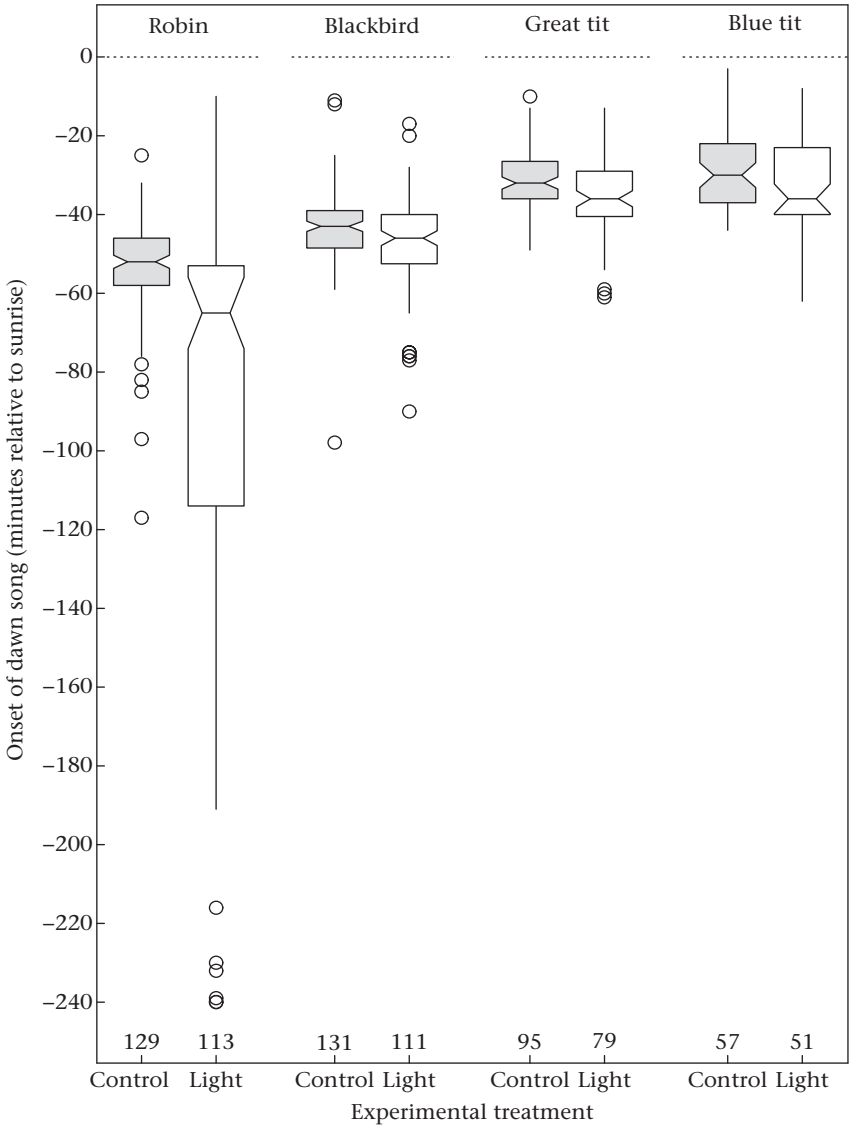

Figure 1. Onset of dawn song relative to sunrise for experimentally illuminated and control days for four songbird species. Data from 2014 and 2015 are pooled. Box plots represent median and upper and lower quartile (horizontal lines), minimum and maximum values (vertical lines, excluding outliers) and outliers (circles represent values outside 1.5 times the interquartile range). Numbers at the bottom indicate sample size (number of mornings during which a dawn chorus was recorded). The dotted horizontal line at the top indicates sunrise.

and 'site' nested in 'year' (two levels) as a random intercept. The fixed effects were 'day of treatment' (from the first to the third night of treatment), 'weather' (in this analysis stormy days were pooled with rainy days because they were restricted to a single cycle) and 'temperature at sunrise'. We ran one-tailed tests because the predicted effect is only in one direction using the pbkrtest package (Halekoh & Højsgaard, 2014); we adjusted P values for multiple testing (eight tests) using the false discovery rate (Benjamini & Yekutieli, 2001). For the robin, for which we found evidence for a carryover effect during the illuminated phase, we evaluated whether it was similar in both years by adding the interaction between 'day of treatment' and 'year' in the model.

Ethical Note

We carried out the experiment on the premises of the Max Planck Institute for Ornithology. Because of the short duration of the manipulation and because no individuals were caught during the course of the study, no permits were required for this work. Disturbance by the experimenter before and during the experimental period was low, because of the small number of visits to each site. Light at night may have caused distress to some birds but, apart from effects on timing of singing, we did not observe any major behavioural changes during the experiment and known breeding attempts within the sites were successful (i.e. fledged chicks). We cannot exclude that the light treatment affected sleep quality, or other aspects of physiology and behaviour, but we designed the treatment such that individuals could move to dark places within their territory to avoid constant exposure to the light and we kept the number of experimental sites to a minimum (see Table A1 for an estimation of the number of affected individuals or breeding pairs).

RESULTS

Effects of Experimental Light Treatment

In all four species, the onset of the dawn chorus was significantly earlier during the experimentally illuminated nights than during the dark control nights (Fig. 1, Table A3). The effect was strongest in robins, which initiated singing on average 37 min earlier during the illuminated mornings ($P < 0.001$), whereas blackbirds, great tits and blue tits initiated singing on average about 4 min earlier during the illuminated cycles (all $P < 0.005$; Table A3).

In the analyses, we controlled for the confounding variables rain (which delayed the onset of dawn singing in all species by 3–5 min, but only significantly so for blue tits; Table A3), the occurrence of a 3-day storm (strong delaying effect of 8–13 min, significant for blackbirds and blue tits; Table A3) and temperature at sunrise (minor, nonsignificant effects, Table A3). The results were generally consistent over the 2 years and between the sites (Figs. 2 and 3 and Fig. A1). Indeed, in the final models (Table A3), the percentage of the total variance in the onset of dawn singing explained by these random factors was low (year: 0% in robin, blackbird and blue tit, 4.8% in great tit; site: 0% in robin, great tit and blue tit, 9.4% in blackbird).

Carryover Effects

In the dark control phase following an illuminated phase, the onset of dawn singing did not progressively become later, as expected if there were carryover effects (the slopes of the onset of singing against day of treatment did not differ significantly from zero for all species; Table 1). In the illuminated phase, the pattern was similar, except for the robin: male robins sang on average 15 min earlier on each consecutive illuminated night (Table 1, Figs. 2a and 3a, b). Note that this effect was mainly restricted to 2015 (interaction between 'day of treatment' and 'year': $t_{66.1} = -1.9$, $P = 0.03$; Fig. 3a, b).

DISCUSSION

Our experiment shows that artificial night lighting caused earlier dawn singing in the four study species (from about 37 min in the naturally earliest singer, i.e. the robin, to 4 min in the blackbird, great tit and blue tit; Fig. 1, Table A3). Because we illuminated a naturally dark habitat in a cyclic fashion, our results suggest that these species, and in particular the robin, can immediately and reversibly adjust their onset of singing to the light conditions (Figs. 2 and 3). We found a delaying effect of rain (and of a 3-day storm) on the onset of singing, as shown previously (Bruni et al.,

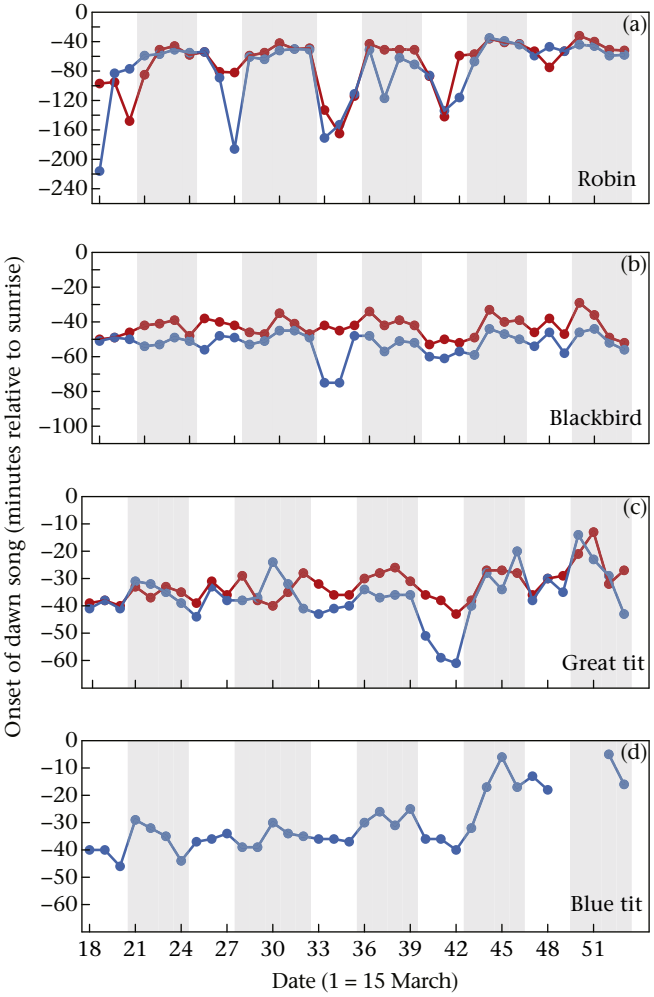

Figure 2. Daily onset of dawn song relative to sunrise during the experimental period in 2014. Data are shown for each species (from the naturally earliest singer (a) to the latest (d)). (a) Robin, (b) blackbird, (c) great tit and (d) blue tit. Each line represents one site (site 1 in red, site 2 in blue). Dark control days are indicated by vertical grey bars. Note the different scale of the ordinate for the different species.

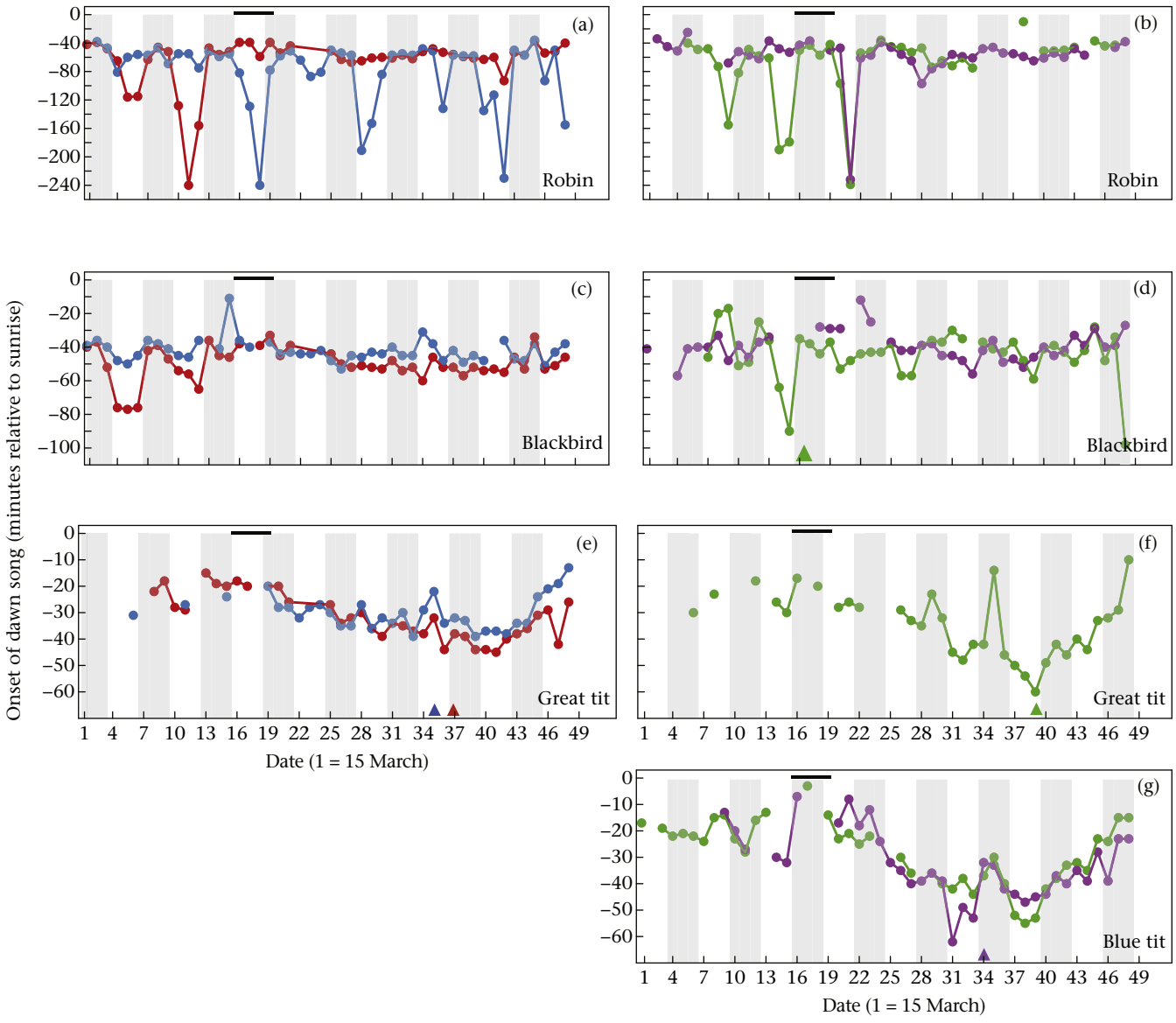

Figure 3. Daily onset of dawn song relative to sunrise during the experimental period in 2015. Data are shown for each species. (a, b) Robin, (c, d) blackbird, (e, f) great tit and (g) blue tit. Each line represents one site: (a, c, e) site 1 in red, site 2 in blue, same as in 2014; (b, d, f, g) site 3 in green, site 4 in purple). Dark control days are indicated by vertical grey bars. Triangles on the x-axes represent the day of the first egg for the breeding individuals (estimated for the blackbird), with colours matching the site where the individuals bred. The solid black line at the top of each graph indicates the occurrence of a severe storm. Note the different scale of the ordinate for the different species.

2014; Da Silva et al., 2014). We found no effect of temperature at sunrise, which again suggests that the timing of dawn song is primarily regulated by light levels (Hutchinson, 2002). We found no evidence for a carryover effect after the light treatment ended, but the robin progressively advanced its start of dawn singing during the light treatment (Table 1, Figs. 2 and 3).

Robins showed the strongest response to the light treatment by singing much earlier in the majority of the illuminated periods than during the dark control nights. Because night lighting was the only manipulated variable at every site, this strongly supports the hypothesis that artificial light at night is the main determinant of nocturnal singing in this species, in accordance with some studies (Da Silva et al., 2014; Kempenaers et al., 2010; Thomas, Drewitt, Kelly, Marples, & Semple, 2003) but in contrast with others (Fuller, Warren, & Gaston, 2007). Interestingly, robins started their dawn song increasingly earlier from the first to the last illuminated night, at least in 2015. This could be a consequence of entrainment of their circadian clock to the change in light regime, due to the pineal gland storing information about past photoperiod (Brandstätter et al., 2000; Gwinner & Brandstätter, 2001). If so, we would also expect that robins would gradually return to their natural time of singing during the dark phase following an illuminated period. However, this was clearly not the case: they immediately returned to the natural times typical of dark forests (Figs. 2a and 3a, b; Da Silva et al., 2014; Kempenaers et al., 2010). This suggests that carryover effects on the start of dawn singing are absent. Of course, the light treatment might still have led to a carryover effect on awakening time (see Schlicht et al., 2014). A similar pattern of light entrainment was found in a study of captive great tits: onset of activity gradually advanced during the illuminated days of the experimental period but it immediately returned to normal during the following control period (de Jong et al., 2016).

Table 1
Results from linear mixed-effects models to test for 'carryover' effects in four songbird species

	Estimate[a]	SE[b]	t[c]	df[d]	P[e]	$R^2_{(m)}$[f]	$R^2_{(c)}$[f]
Robin							
Control period							
Intercept	−58.9	2.6					
Day of treatment	1.6	1.1	1.5	55.1	0.3	0.23	0.47
Illuminated period							
Intercept	−62.0	13.0					
Day of treatment	**−15.1**	**4.8**	**−3.2**	**34.1**	**0.01**	**0.08**	**0.39**
Blackbird							
Control period							
Intercept	−43.2	2.5					
Day of treatment	−0.8	0.9	−0.9	30.8	0.9	0.08	0.35
Illuminated period							
Intercept	−47.7	3.2					
Day of treatment	−0.6	0.9	−0.7	17.6	0.7	0.04	0.54
Great tit							
Control period							
Intercept	−31.1	2.1					
Day of treatment	−0.4	0.8	−0.5	40.1	0.9	0.10	0.42
Illuminated period							
Intercept	−35.0	2.4					
Day of treatment	−0.3	0.6	−0.4	13.9	0.7	0.02	0.78
Blue tit							
Control period							
Intercept	−29.0	3.1					
Day of treatment	0.4	0.9	0.5	5.6	0.9	0.10	0.82
Illuminated period							
Intercept	−33.7	3.1					
Day of treatment	1.0	0.8	1.2	3.8	0.9	0.06	0.92

The table shows effects of day of treatment (separately for each treatment type) on the onset of dawn singing. A carryover effect would lead to a positive slope after a light treatment (onset of singing should become progressively later during the control period), and a negative slope after a control period (onset of singing should become progressively earlier during the illuminated period). We controlled for 'weather' and 'temperature at sunrise' in the models.

[a] Effect size (in minutes relative to sunrise).
[b] Standard error.
[c] One-tailed tests.
[d] The degrees of freedom were computed using the Kenward–Roger approximation (Halekoh & Højsgaard, 2014).
[e] P values were corrected for multiple testing (eight tests) using the false discovery rate.
[f] $R^2_{(m)}$: proportion of the variance explained by the fixed effects; $R^2_{(c)}$: proportion of the variance explained by both fixed and random effects.

For the robin, the estimated effect size in response to the experimental treatment (37 min; Table 1) was higher than the effect size previously reported in a correlational study conducted in the same general area (Da Silva et al. 2014: mean and 95% CI: 19 min [9–29 min]), However, another correlational study showed an advance in the start of singing of 64 min under artificial light conditions (Kempenaers et al., 2010), and we note that in Da Silva et al. (2014) some robins started singing before the recordings started (1.5 h before sunrise). For the other three species, the estimated effect size in our experiment was 4 min (Table 1), which is markedly lower than the effects reported in Da Silva et al., 2014 (blackbird: 19 min [11–27 min]; great tit: 21 min [11–31 min]; blue tit: 15 min [5–25 min]). For the blackbird and the great tit, but not for the blue tit, these values are also clearly lower than those reported in Kempenaers et al., 2010 (blackbird: 41 min; great tit: 32 min; blue tit: 3 min).

Although birds in our study were exposed to light only during a short period of the year, and only on some days (unlike urban birds), the results are consistent across the 2 experimental years, which suggests that the timing of dawn singing in blackbirds and tits may not be as plastic as that in the robin. The earlier singing of these species observed in illuminated urban habitats may therefore be due to a combination of an initial moderate plasticity in singing behaviour, together with longer-term effects such as learning, a pronounced circadian phase shift, and developmental or even genetic effects. Such long-term effects are particularly relevant in sedentary species that are exposed to light year round (e.g. urban blackbirds, Partecke & Gwinner, 2007). Alternatively, other variables correlated with the presence of artificial night lighting, such as noise pollution (Fuller et al., 2007; Nordt & Klenke, 2013, but see; Da Silva et al., 2014) and temperature (Taha, 1997), may have amplified the light effect in the correlational studies. It is also conceivable that settlement patterns are phenotype dependent, such that early singers are more likely to end up in territories affected by artificial night lighting. Genetic changes in the timing of singing in response to light pollution are generally plausible (high heritability of circadian period length in great tits, Helm & Visser, 2010; faster circadian rhythms in urban versus rural blackbirds, Dominoni, Helm, Lehmann, Dowse, & Partecke, 2013), but are unlikely to explain our previous correlational results because the birds in the illuminated and dark areas were usually from the same population. Nevertheless, a moderate behavioural plasticity (as observed here) may facilitate subsequent micro-evolution in a novel illuminated habitat, by permitting a phenotype closer to the new adaptive peak, which can then be followed by directional selection favouring extreme phenotypes (genetic assimilation; Price et al., 2003). In contrast, rural robins colonizing novel illuminated habitats might adapt immediately to artificial night lighting, given the strong behavioural plasticity observed in our experiment.

We found that the plasticity was limited, especially in blackbirds and great tits, and perhaps also in blue tits. However, we cannot exclude that plasticity may have been underestimated in our experimental set-up, for the following reasons. First, inspection of Figs. 2 and 3 suggest that light triggered earlier singing in these species only during part of the experiment, perhaps related to the

peak fertility of each species (Fig. 3). Blackbirds and several tit species naturally show the earliest dawn singing during peak female fertility (Cuthill & Macdonald, 1990; Dominoni & Partecke, 2015; Mace, 1987; Welling, Koivula, & Lahti, 1995) and the response to light may be strongest at this time, as suggested previously (Da Silva et al., 2014; Dominoni & Partecke, 2015; Dominoni et al., 2014; Kempenaers et al., 2010). However, we did not find a significant effect of the interaction between date and light in our experiment, which does not support this claim.

Second, the blackbirds and great tits in our experiment may have been more prone, accidentally or intentionally, to avoid the illuminated areas. Although behavioural observations and analysis from the two recorders at each site in 2015 showed that the focal males were singing at or near the illuminated sites during most mornings, birds may have roosted further away from the illuminated area. Dominoni et al. (2014) reported that urban blackbirds roosted under levels of light that were at least 20-fold lower than those of the surrounding streetlights. Alternatively, birds may have roosted at the illuminated site, and may have been awake earlier due to the light, but may actively have chosen not to start singing. This choice may be conditional upon factors such as social stimulation (number of singing males; Foote, Fitzsimmons, Mennill, & Ratcliffe, 2011), body condition (Cuthill & Macdonald, 1990; Grava et al., 2009; Russ et al., 2015), personality (Naguib, van Rooij, Snijders, & van Oers, 2016), stress levels (Russ et al., 2015) or predation risk (Schmidt & Belinsky, 2013). The latter may play a role in our study, because night predators (owls) may be more numerous in forests than in cities (although evidence is equivocal, see Chamberlain et al., 2009), and are indeed common at the study site. Moreover, forest blackbirds show higher stress responses than urban blackbirds (Partecke, Schwabl, & Gwinner, 2006), which may have reduced their response to the light.

Third, although light was the only manipulated variable, it may have caused other changes in the environment (e.g. insect abundance, attraction or repellence of small mammals or birds of prey; Spoelstra et al., 2015), thereby indirectly influencing bird behaviour through effects on e.g. predation risk, roosting or body condition.

Fourth, because our light treatment was at a relatively small, local scale and because we do not know exactly where birds were singing, we cannot exclude that some of the individuals we recorded were in fact not affected by the light. This would lead to an underestimate of the effect of light on timing of singing. Lastly, given the relatively small sample size of our study (in terms of number of individual birds), it is possible that the true mean effects of light are larger than the estimated means, simply by coincidence.

Conclusion

Behavioural plasticity is generally assumed to help species adapt to anthropogenic changes (Price et al., 2003; Sih et al., 2011). Previous work has shown that plastic adjustments of singing behaviour may help songbirds deal with noise pollution (Arroyo-Solís, Castillo, Figueroa, López-Sánchez, & Slabbekoorn, 2013; Gross, Pasinelli, & Kunc, 2010; Schuster, Zollinger, Lesku, & Brumm, 2012). Our results demonstrate that artificial night lighting causes an earlier start of the dawn chorus in four songbird species, and suggest that behavioural plasticity is also an important mechanism underlying early singing in habitats exposed to light pollution. On a broader scale, further work should determine whether other song characteristics (e.g. song consistency, repertoire size, total song output), which can signal quality in some species (e.g. Rivera-Gutierrez, Pinxten, & Eens, 2010), are also affected by light pollution. More work is also needed to determine whether the plastic response to light in terms of timing of singing (and possibly other factors) is adaptive or not. Anthropogenic effects (e.g. artificial light) can imitate ancestral cues (natural light) and animals exposed to this new environment may change their behaviour with innate responses that are no longer adaptive (Blumstein & Berger-Tal, 2015). Early singing may for example lead to sleep deprivation (Raap, Pinxten, & Eens, 2015) or to increased stress levels (Ouyang et al., 2015). Further work should also investigate whether songbirds can lower these potential costs by using (adaptive) compensatory behaviours (Sih et al., 2011) such as light avoidance, increased foraging, or decreased song output later during the day.

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APPENDIX

Table A1
Information about presence and breeding of the study species at each site in 2014 and 2015

		2014		2015			
		Site 1	Site 2	Site 1	Site 2	Site 3	Site 4
Robin	Present	Yes	Yes	Yes[a]	Yes[a]	Yes[a]	Yes[a]
	Paired	?	?	Yes	No	Yes	Yes
	Breeding	?	?	No	No	No	No
Blackbird	Present	Yes	Yes	Yes	Yes	Yes	Yes
	Paired	?	?	No	Yes	Yes	No
	Breeding	?	?	No	No	Yes	No
Great tit	Present	Yes	Yes	Yes	Yes	Yes[b]	*No*[c]
	Paired	?	?	Yes	Yes	Yes	*No*
	Breeding	?	?	Yes	Yes	Yes	*No*
Blue tit	Present	No	Yes	*No*	*No*	Yes	Yes
	Paired	No	?	*No*	*No*	Yes	Yes
	Breeding	No	?	*No*	*No*	No	Yes

In 2014, only information from the recordings was available. In 2015, we conducted behavioural observations and installed three nestboxes (dimensions: 18 × 15 cm and 27 cm high; entrance hole: 32 mm) at each site in mid-February to obtain information on the timing of breeding of great or blue tits. We checked all nestboxes at least weekly during the experiment, and we also searched for robin and blackbird nests. 'Present' indicates that at least one male was observed/recorded regularly singing at the site during the course of the experiment. A superscript indicates two males were regularly singing at the site. 'Paired' indicates that a female was observed with the singing male at the site at least once. For the robin, sex determination was based on behaviour (i.e. presence of alarm-calling females during the male dawn chorus). 'Breeding' indicates that the pair was known to have bred at the site, based on the observation of eggs, either in a nestbox (blue and great tits) or in an open nest (blackbird). All known breeding attempts led to successful hatching and fledging. Great tits nested in all sites except 4, whereas the blue tit only nested in site 4 but was consistently observed also in site 3. We therefore excluded data on great tits from site 4 and data on blue tits from site 1 and 2 from the analysis (as shown in italics in the table).

[a] In sites 1, 2 and 3, one focal male sang within the illuminated area and one peripheral male sang at the edge of the area. In site 4, only one focal male was present, but he was often observed singing at the periphery or outside the illuminated area.

[b] Two focal males sang at the site during most of the experimental period. We did not discriminate between them in the sound analysis.

[c] One great tit pair attempted to settle and breed at the periphery of the site in mid-April (in a neighbouring nestbox), but deserted after one egg was laid.

Table A2
Model selection using AIC

Species	Candidate models	AICc	ΔAICc	$R^2_{(m)}$	$R^2_{(c)}$
Robin	**Date+Treatment**	173.9	0.0	0.55	0.56
	Date*Treatment	174.3	0.4	0.22	0.28
Blackbird	$(Date)^2$*Treatment	1709.5	0.0	0.13	0.32
	$(Date)^2$+Treatment	1711.0	1.5	0.11	0.31
Great tit	**$(Date)^2$+Treatment**	1110.2	0.0	0.39	0.46
	$(Date)^2$*Treatment	1111.3	1.1	0.39	0.46
Blue tit	$(Date)^2$*Treatment	695.5	0.0	0.57	0.57
	$(Date)^2$+Treatment	697.4	1.9	0.56	0.56

The AICc measures the relative quality of a candidate model, adjusted for the sample size. ΔAICc is defined as the difference in AICc between the best model (with lowest AICc) and a competing candidate model. When $\Delta AIC_c > 2.0$, the difference between the two competing models is substantial (Burnham & Anderson, 2002). When $\Delta AIC_c < 2.0$, the most parsimonious model was selected (final models are indicated in bold). We also present R^2 values for each candidate model. $R^2_{(m)}$ gives the variance explained by the fixed effects, while $R^2_{(c)}$ gives the variance explained by both fixed and random effects.

Table A3
Results from the final linear mixed-effects models showing effects of experimental light treatment, date, weather and temperature on the onset of dawn singing in four songbird species

	Estimate[a]	SE[b]	z^c	P
Robin				
Intercept	−58.8	2.6		
Light[d]	**−37.1**	1.7	−7.2	<0.001
Date	1.0	1.0	0.8	0.9
Rain[e]	4.5	1.8	2.5	0.06
Storm[f]	8.3	3.6	1.7	0.4
Temperature	1.0	1.1	0.3	1.0
Blackbird				
Intercept	−46.2	2.2		
Light[d]	**−3.8**	1.2	−3.2	0.01
Date	−20.4	13.0	−1.6	0.6
$(Date)^2$	−7.1	11.8	−0.6	1.0
Rain[e]	3.3	1.4	2.3	0.1
Storm[f]	**9.2**	3.2	2.9	0.03
Temperature	0.4	0.2	2.0	0.3
Great tit				
Intercept	−32.9	1.6		
Light[d]	**−4.1**	0.9	−4.5	<0.001
Date	−11.5	13.2	−0.9	1.0
$(Date)^2$	**32.5**	11.2	2.9	0.02
Rain[e]	2.8	1.2	2.4	0.1
Storm[f]	7.6	2.9	2.6	0.06
Temperature	0.2	0.2	0.8	0.9
Blue tit				
Intercept	−30.2	2.4		
Light[d]	**−3.8**	1.1	−3.4	0.005
Date	−16.0	19.9	−0.8	1.0
$(Date)^2$	**64.3**	17.4	3.7	0.002
Rain[e]	**5.3**	1.4	3.8	0.001
Storm[f]	**13.2**	3.8	3.5	0.003
Temperature	0.2	0.2	0.9	1.0

[a] Effect size (in minutes relative to sunrise).

[b] Standard error.

[c] Sample sizes: robin: 242; blackbird: 242; great tit: 174; blue tit: 111.

[d] Estimates are for illuminated days compared to control days.

[e] Estimates are for rainy days compared to clear days.

[f] Estimates are for stormy days compared to clear days.

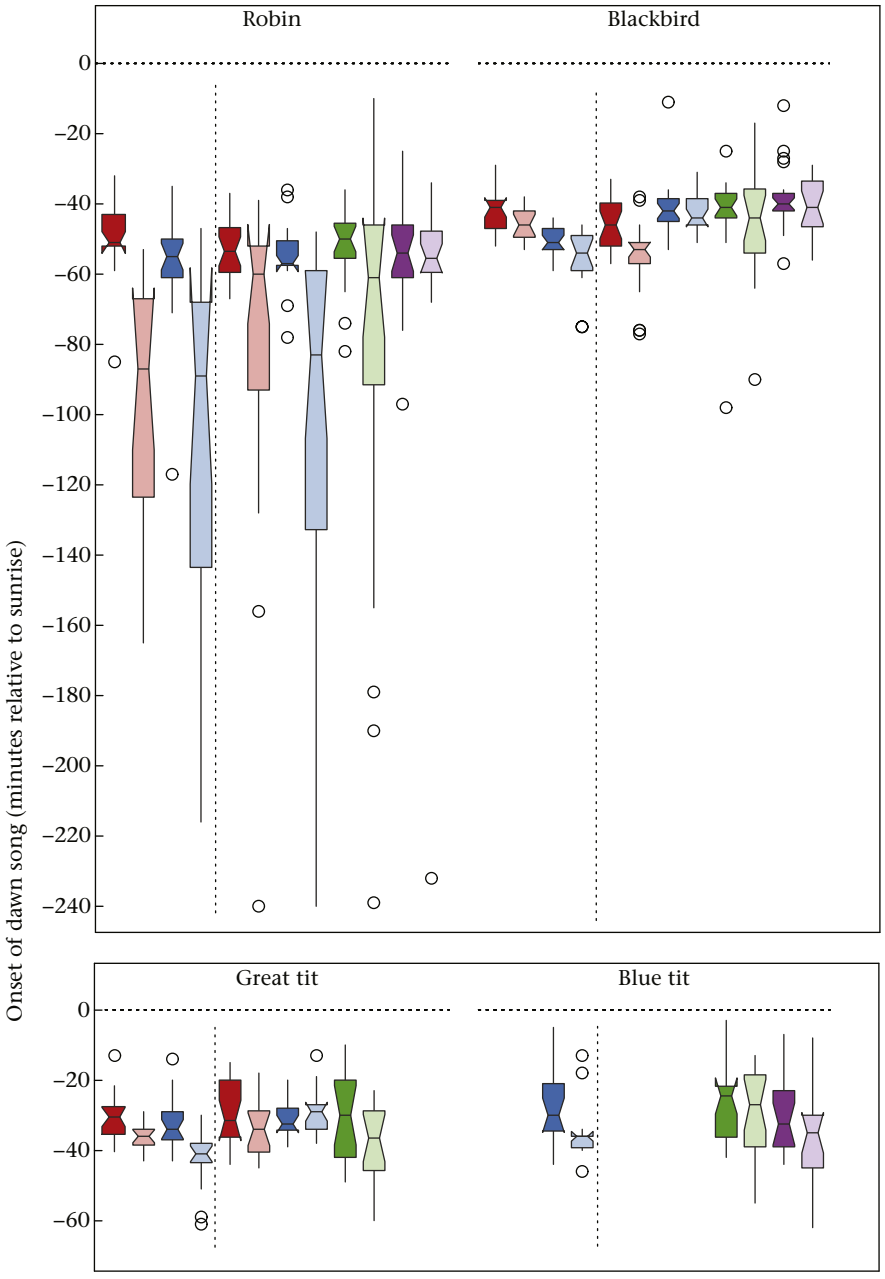

Figure A1. Onset of dawn song relative to sunrise for four songbird species in control (naturally dark) and experimental (artificial night lighting) conditions for each site and year separately. Box plots indicate median, upper and lower quartile (horizontal lines), minimum and maximum values (vertical lines, excluding outliers) and outliers (circles represent values outside 1.5 times the interquartile range). Each pair of boxes represents one site (site 1 in red, site 2 in blue, site 3 in green, site 4 in purple; same colours as in Figs. 2 and 3 in the main text). Intense colours represent the control situation, light colours the experimental situation. For each species, a dotted vertical line separates the two sites used in 2014 (on the left) from the four sites used in 2015 (on the right). Sample sizes (number of mornings during which a dawn chorus was recorded) can be obtained from Figs. 2 and 3. The dotted horizontal lines indicate sunrise.